Earthworm Effects on Soil Structure with Emphasis on Soil Stability and Soil Water Movement

S. Schrader, M. Joschko, H. Kula, and O. Larink

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I. Introduction

Earthworms affect the soil by burrowing through the substrate, either by pushing soil aside or by ingesting soil and egesting it as casts. Thus formed burrows, which can vary in shape and stability, may provide preferential flow paths for water and solutes. The ingested and egested soil is changed through the passage in the intestine. The alterations are reflected in the function of the processed soil.

In order to understand earthworm effects on the soil fabric it is essential to combine soil physical and soil morphological measurements. Soil physical measurements are feasible to quantify the earthworm activity in terms of general soil functions like water movement and stability. The morphological analysis

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gives insight into the mechanisms by which earthworms influence soil. The challenge therefore is 1) to identify physical parameters by which the soil fabric influenced by earthworms may be characterized functionally; and 2) to find or develop suitable techniques by which the earthworm affected soil can be studied morphologically. In the past there has been a lack especially in techniques to analyze soil morphologically. With the help of techniques like fluorescence microscopy and X-ray computed tomography, tools are now available to analyze the earthworm affected soil fabric adequately in a quantitative way.

The improved stability of earthworm casts has been stressed by various authors (Darwin 1881, McKenzie and Dexter 1987, Marinissen and Dexter 1990). The number of studies trying to correlate soil stability measurements and micromorphological analyses still is very limited (Shipitalo and Protz 1989). The technique of fluorescence microscopy in combination with staining techniques was pushed forward by Altemüller and van Vliet-Lanoe (1990). A combination with soil stability measurements has not been investigated yet. First results of this approach are presented here.

In several field studies the earthworm activity was correlated with higher infiltration rates (Ehlers 1975, Fallahi 1978). Direct contributions of earthworm channels to the water movement under natural conditions have been proved by several authors (Edwards et al. 1989, Urbanek and Dolezal 1992). The earthworm burrows in these studies were produced by the anecic earthworm species *Lumbricus terrestris*. Their burrows are continuous, deep-reaching channels and can therefore be analyzed functionally by pouring liquid preparations (e.g., gypsum) on the surface (Bouma et al. 1982) or by tapping it from below the soil surface (Edwards et al. 1989). A lack of information exists with respect to the hydraulic effects of burrows produced by endogeic earthworms. This group comprises soil feeding species like *Aporrectodea caliginosa* and *Octolasion cyaneum* and represents the most important earthworm group of agricultural sites. These species produce burrows of varying continuity (Kretzschmar 1982). A problem has been to assess the burrows morphologically. In some laboratory studies the burrows on the outer walls of transparent soil columns were analyzed (Kretzschmar 1990, Joschko et al. 1992). In the past the three-dimensional structure of the burrows was assessed by excavating them (Kretzschmar 1982, Lighthart et al. 1993). X-ray computed tomography proved to be a suitable technique to characterize these burrows morphologically in three dimensions even when their continuity is low (Joschko et al. 1991).

An important step to the functional and morphological assessment of biological structures in the field are pot experiments. Despite the disadvantages connected with the simplification of the field situation they offer several advantages: a) earthworm affected soil can easily be identified and related to earthworm biomass and species; b) new methods of morphological analysis can be tested with sieved, artificially compacted soil or undisturbed natural soil cores.

This chapter presents results from experiments with soil filled columns and planar glass cuvettes inoculated with the following earthworm species:

Lumbricus terrestris L. 1758, *Aporrectodea caliginosa* (Sav. 1826), *Aporrectodea longa* Ude 1885 and *Octolasion cyaneum* (Sav. 1826). The aim of this study was 1) to measure the stability of casts and burrow wall material and to relate it to its morphological and chemical structure, and 2) to characterize burrows of various earthworm species morphologically and functionally with respect to water movement.

II. Analysis of Earthworm Altered Soil

A. Introduction

This part of the paper describes predominantly soil genetical aspects caused by the burrowing activity of earthworms. Their contribution to soil formation depends on the burrowing behavior (Bouché 1977) and food selection (Lee 1985) of the different species. The anecic species *L. terrestris* is detritivorous and was fed with dried leaves of *Tilia vulgaris*. *A. longa* is also an anecic species but geophagous like *A. caliginosa*. Both were fed with dried pulverized cattle manure.

The daily cast production per gram live weight of endogeic and anecic earthworms was determined in a laboratory experiment (Schrader 1993a). Planar glass cuvettes (Schrader and Joschko 1991) were used for the experiments reducing the soil volume to approximately 2 dimensions. Thus, it was possible to study the whole burrow system. Following Altemüller and Vorbach (1987) each vessel was filled with sieved soil aggregates moistened to 17 wt.%. A homogeneously aggregated substrate allows a definite discrimination between soil and earthworm casts. The bulk density ranged from 1.2-1.4 g/cm^3. The vessels inoculated with one species each were placed vertically in a dark room at 12°C.

Results are shown in Figure 1 for *A. caliginosa* and *A. longa* for a period of almost 30 days. With a daily cast production of 12.3 g per gram live weight *A. caliginosa* egested twice as much as *A. longa*. This high soil turnover is essential for endogeic geophagous species because they have to satisfy their energy supply by digesting microorganisms and organic matter present in the soil matrix. From this point of view *A. caliginosa* plays an important role in the formation of soil structure.

The potential cast production under natural conditions was estimated by correlating data from the laboratory studies with data from several field studies. Table 1 shows the extrapolated cast production of *A. caliginosa* and *A. longa* in arable land. The three field study sites are characterized by the same soil type (luvisol FAO) and a bulk density similar to the soil used in the laboratory experiments (1.4 g/cm^3). Earthworms were collected with activity related expulsion methods.

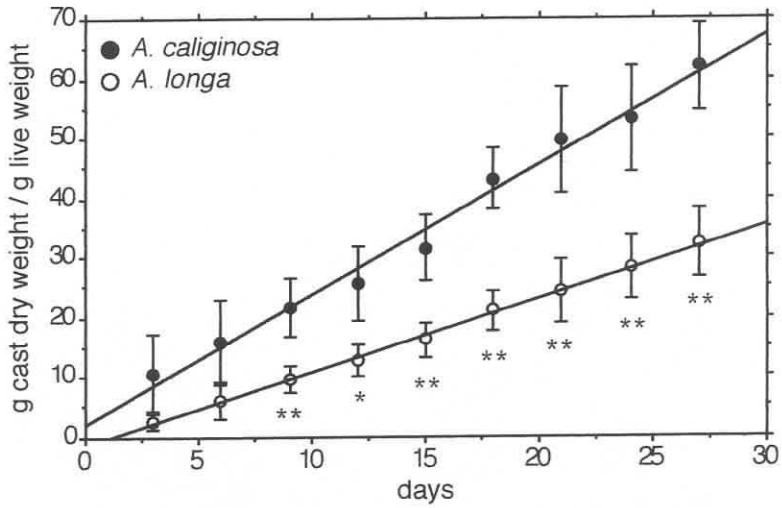

Figure 1. Temporal development of cast production. Arithmetic means (±SD) for n=4 vessels with 2 individuals each. *P≤0.05 and **P≤0.01. *A. caliginosa*: y = 2.17x + 2.22; r^2=0.988; *A. longa*: y = 1.23x - 1.30; r^2=0.998.

Table 1. Extrapolation of cast production data from laboratory to field conditions, calculated by biomass data from field studies.

	----------*A. caliginosa*----------		----*A. longa*----
	Poier and Richter, 1992	Söchtig, 1992	Knüsting, 1992
live biomass of adults	35 kg ha^{-1}	64 kg ha^{-1}	16 kg ha^{-1}
cast production (Schrader, 1993b)	13.1 t ha^{-1} yr^{-1*}	23.8 t ha^{-1} yr^{-1*}	3.1 t ha^{-1} yr^{-1*}

* calculated for an average earthworm activity in temperate regions of 5 months per year.

B. Fluorescence Microscopy

Thin sections were prepared to study the influence of geophagous earthworms on microflora and fabric of the soil during intestinal passage. Ingested and egested soil was compared to the surrounding soil. The investigation was conducted with four adult individuals of *A. caliginosa*. After burrowing in a sieved luvic gleysol, FAO (Mull-Pseudogley, weak loamy silt, 18% clay,

fraction 0.5-1.0 mm) for 8 days the earthworms were anesthetized by CO_2-fumigation in situ and fixed together with the surrounding soil in 2.5% glutaraldehyde. The samples were dehydrated by a graded series of aceton and impregnated with Vestopal-resin (Schrader and Altemüller 1993). For details about thin section preparation see Altemüller (1974). Staining occurred with thiazine red prior to dehydration and embedding (Altemüller and van Vliet-Lanoe 1990). The walls of the body and the gut functioned as barriers against thiazine red as the fluorescence of the gut content was low. For this reason some parts were counterstained additionally either with berberine sulfate or brilliant sulfoflavine directly to the prepared thin sections. They were examined by fluorescence microscopy with incident light excitation.

The ingested and molded soil revealed a specific fabric in comparison to the surrounding soil. At low magnification mineral grains of the silt and fine sand fraction which were coated with clay in part and debris of plant residues were conspicuous. With increasing magnitude and after counterstaining with berberine sulfate a characteristic arrangement of the finer textured material became evident. Clayey microaggregates of 2-6 μm size were among the coarser grains (Figure 2). These microaggregates were arranged spaciously and loosely probably due to intestinal mucous liquid. They represented the most frequent element of the fabric of the gut content. Sporadically undestroyed aggregates maintaining the primary compact fabric of the uningested soil (Figure 3) passed the intestine. This was also observed by Joschko and Altemüller (1989) and Altemüller and Joschko (1992) in casts of *L. terrestris*. The microaggregates have angular edges, i.e., the faces of the aggregates accommodate each other. This shape indicates that the microaggregates originated from larger compact clay units which were broken into fragments by peristaltic motion of the intestinal muscles and uneven swelling.

After counterstaining with brilliant sulfoflavine, rod-shaped and coccal microorganisms became well contrasted. The rod-cells were 5-15 μm long and clustered adjacent to organic matter. Before ingestion only coccal microorganisms were found in the soil. Thin sections of the hind gut showed exclusively rod-cells but no coccal cells. From this observation it can be concluded that the coccal cells are digested during gut passage of the ingested soil whereas the rod-cells may be part of the symbiotic gut flora. They were almost absent in the egested soil. The distribution of the microorganisms was more dense in the non-ingested soil than in the intestine.

The fabric of the casts was compact like the aggregates of the surrounding soil. This compaction is explained by a lower water content in the casts because most of the intestinal liquid can be reabsorbed in the hind gut (Barois and Lavelle 1986). Cross sections showed a successive colonization of the casts by microorganisms. A decreasing gradient of coccal cells from the surface to the inner part could be observed. This fact obviously has consequences for the aggregate stability of the casts which is dealt with in the following chapter.

The burrow-wall is lined by a membranous mucus layer which fluoresced brightly. A dense community of microorganisms lived in the drilosphere (sensu

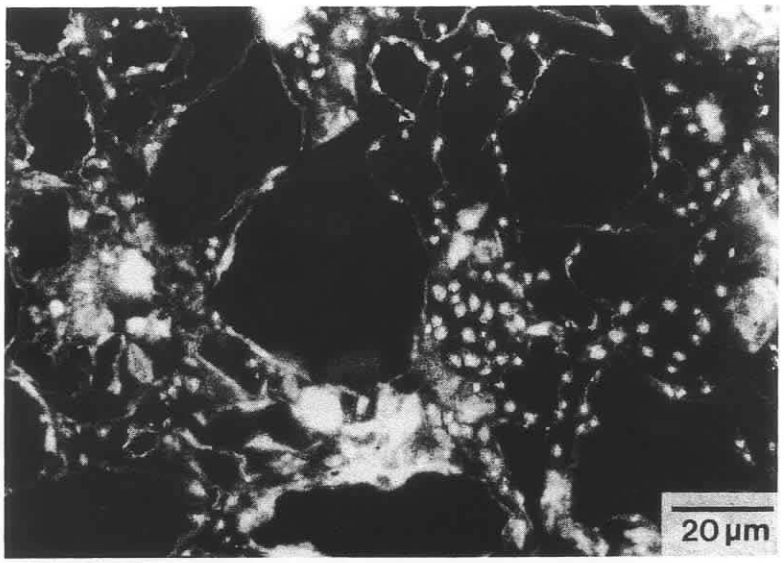

Figure 2. Thin section of the intestinal content of *A. caliginosa*. Clayey microaggregates of fragmental character were loosely arranged among silt grains as a result of mucous liquid. The clay material is contrasted by counterstaining with berberine sulfate.

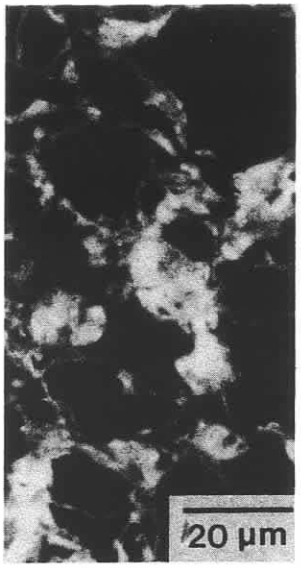

Figure 3. Thin section of the bulk soil. The internal fabric of the aggregates is compact showing silt grains bonded within clay material. The clay material is contrasted by counterstaining with berberine sulfate.

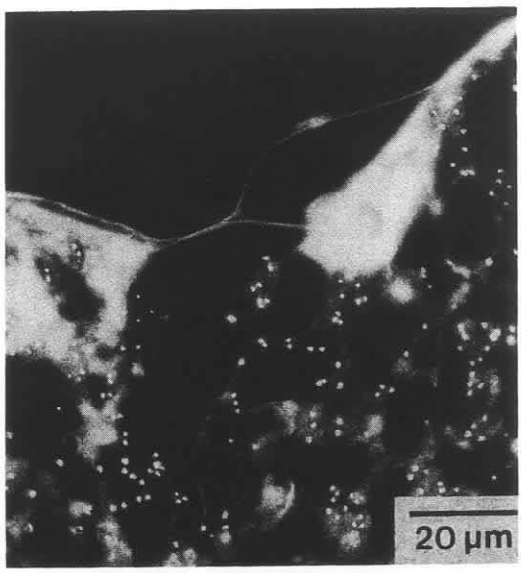

Figure 4. Cross section of the burrow-wall of *A. caliginosa*. The sector shows the lining membranous mucus layer and a dense community of microorganisms which both fluoresced brightly.

Bouché 1975) which is a mixture of mucus, casts and soil. These characteristics are presented in Figure 4.

C. Soil Physical and Chemical Measurements

Selected soil physical and chemical properties were measured to characterize the influence of earthworms on soil aggregate stability. Adult individuals of *A. caliginosa, A. longa* and *L. terrestris* were inoculated in planar glass cuvettes which were filled with sieved aggregates of an orthic luvisol, FAO (Parabraunerde, loamy silt, fraction 1-2 mm). After 4 weeks the cuvettes were opened, the earthworms removed and the soil dried in situ. Afterwards pure casts were collected from the soil surface and burrow wall material was separated from soil aggregates. Casts and burrow-wall material were cautiously broken into 1-2 mm aggregates (Hartge and Horn 1989). Two kinds of soil aggregates served as control: 1) 1-2 mm natural aggregates as used as substratum; 2) remolded soil aggregates. The soil was kneaded at a water content of about the liquid limit (ca. 30 wt.%) to simulate the intestinal passage. Afterwards it was air-dried and broken into 1-2 mm sized aggregates.

The aggregate water stability (n=5) was determined following the method of Kemper and Rosenau (1986). The tensile strength of air-dry (ca. 60% humidity)

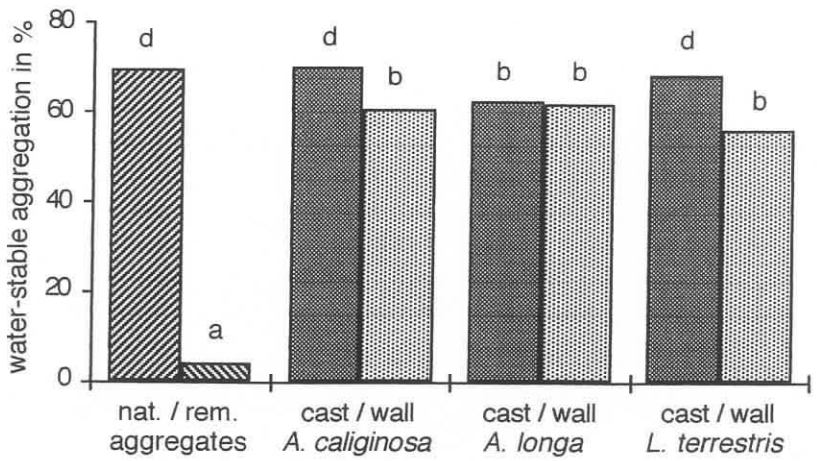

Figure 5. Arithmetic means of water-stable (n=5) of natural (nat.) and remolded (rem.) aggregates, casts, and burrow-wall material of the earthworms; bars with different letters are significantly different at 5% level using Tukey's multiple t-test. (From Zhang, H. and Schrader, S. *Biol. Fertil. Soils* 15, 229-234, 1993. With permission.)

aggregates was measured by indirect tension with the compression test apparatus WF 10020 from Wykeham Farrance, England. The aggregates were placed between two metal plates and crushed with a velocity of 0.38 mm/min. The crushing forces were determined with an electrical balance. The tensile strength of individual aggregates (n=50) was calculated using the equation of Dexter and Kroesbergen (1985).

The total carbon content (n=3) was determined by dry combustion of 0.20 g soil in a furnace using a Leco CR-12 carbon system (Leco Corp., Michigan, USA). In addition, the organic carbon content was determined in samples from which carbonate had been removed with 10% HCl. The difference of both results was calculated to get information about the carbonate content suggesting $CaCO_3$ as the main carbonate compound. The monomeres of the polysaccharides and their content in all samples were determined using the high performance anion-exchange chromatography coupled with pulsed amperometric detection according to Martens and Frankenberger (1990). The method was modified into a 2-step extraction procedure as described by Zhang and Schrader (1993). Acid-extractable polysaccharides which are tightly bonded and water-extractable polysaccharides were detected separately.

The results of the wet-sieving procedure are presented in Figure 5. The most evident difference was measured within the control. In contrast to 70% of the natural aggregates less than 5% of the remolded ones were water-stable. Compared to natural aggregates the stability of casts and burrow-wall material was preserved or only slightly reduced. ANOVA shows that casts of *A. caliginosa* and *L. terrestris* were significantly more water-stable than their

burrow-wall but the data were the same in natural aggregates. No difference was determined between casts and burrow-wall of *A. longa* but the stability was reduced significantly.

The measurement of the tensile strength also showed the greatest difference between natural (468 kPa) and remolded (264 kPa) aggregates. Casts and burrow-wall of *Aporrectodea spp.* were significantly less stable than natural aggregates but more stable than remolded aggregates. The samples of *L. terrestris* preserved the stability of the natural aggregates. Differences between casts and burrow-wall of the same species were not significant.

Total carbon content was increased by 20-38% in casts and by 3-18% in the burrow-wall. The increase in organic carbon was 21-43% in casts and 4-21% in the burrow-wall compared to bulk soil. With respect to the earthworm species the increase in total and organic carbon contents is in the following order: *L. terrestris* > *A. caliginosa* > *A. longa*. The carbonate content of the natural soil was about 0.63% while that of the casts and burrow-wall material from *L. terrestris* was reduced to about 0.3%. In contrast the carbonate content of soil influenced by *A. caliginosa* was increased by more than 0.4% while that from *A. longa* showed no noticeable changes. There were only slight differences between casts and burrow-wall.

With respect to the remolded aggregates and the earthworm influenced soil there is a significant correlation between tensile strength and the C_{org} content on one hand and between tensile strength and the $CaCO_3$ content on the other hand (Figure 6). The tensile strength increases with increasing C_{org} concentration and decreasing carbonate concentration. According to Shipitalo and Protz (1989) this result may refer to the formation of clay-polyvalent cation-organic matter linkages. After Hayes (1983) the formation of Ca-humate is an important process during digestion in earthworms. The organic carbon content is also positively correlated with the water stability ($p \leq 0.05$).

Although the polysaccharide content increased in casts and burrow-wall material no statistically significant correlation could be found, neither in relation to tensile strength nor to water stability. The percentage of acid-extractable polysaccharides amounted to 69-87% of the total content. In comparison to bulk soil the total polysaccharide content of casts increased to 35% with *L. terrestris*, to 74% with *A. caliginosa* and to 87% with *A. longa*. Half of this increase was measured in the burrow-wall of both *Aporrectodea* species. This result could be explained by an enrichment of mucopolysaccharides in the casts resulting from digestion. In the geophagous *Pontoscolex corethrurus*, Lavelle et al. (1983) detected a much lower mucus content in the casts compared to the intestine but it was increased in relation to non-ingested soil. In *L. terrestris* the polysaccharide content of burrow-wall material was increased to 46%, i.e. 11% more than that detected in the casts. We presume in this case a stronger enrichment of polysaccharides from cutaneous than from intestinal mucus because *L. terrestris* lives as an anecic species in only one vertical burrow. All secreted cutaneous mucus is absorbed by the walls of this single burrow. Furthermore, in contrast to *A. longa,* litter fragments line the walls which enhance microbial activity and

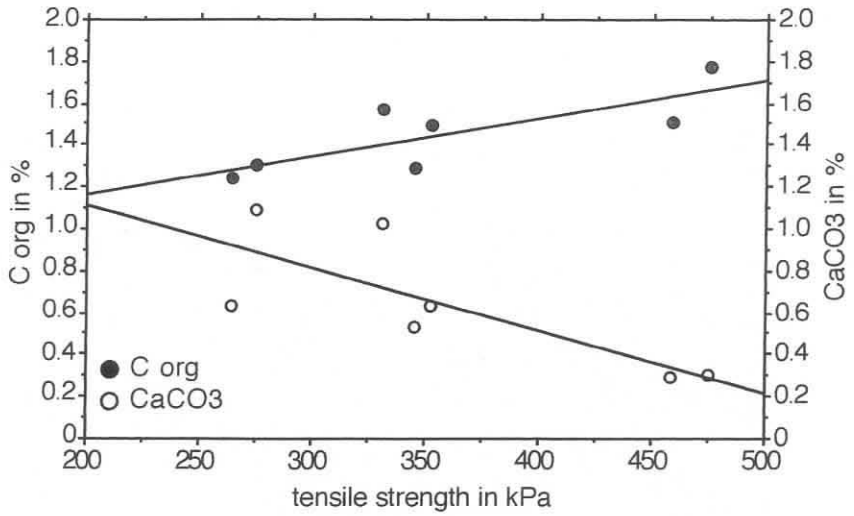

Figure 6. Arithmetic means of tensile strength (n=50), organic carbon content (n=3), and calcium carbonate content (calculated) of remolded aggregates, casts and burrow wall material. Correlation between tensile strength and C_{org}: $y = 0.802 + 0.002x$; $r^2=0.628^{***}$. Correlation between tensile strength and $CaCO_3$: $y = 1.706 - 0.003x$; $r^2=0.593^{***}$. $P \leq 0.001$. (From Zhang, H. and Schrader, S. *Biol. Fertil. Soils* 15, 229- 234, 1993. With permission.)

the formation of microbial polysaccharides. With respect to the polysaccharide composition glucose is the main component (ca. 50%), followed by galactose, glucosamine and 4 other monosaccharides. Galactosamine, mannose and rhamnose amounted to 15-20% in total. Arabinose was detected only in samples of *L. terrestris* with less than 1%. No uncombined monosaccharides were detected in the non-hydrolized extracts.

III. Analysis of the Burrow System

A. Introduction

The natural soil body enables earthworms to move in all three dimensions. A realistic assessment of earthworm burrows and their effect on soil water movement would therefore have to deal adequately with the three-dimensionality. X-ray computed tomography proved to be a useful tool for the three-dimensional assessment of earthworm burrow systems (Joschko et al. 1991). The principle of X-ray computed tomography was described by Hounsfield (1980). The procedure of the three-dimensional reconstruction was published in detail by Joschko et al. (1991). The reconstruction yields a visualization of the three-dimensional burrows and allows a quantification by summing up the respective picture elements (pixels) in rows or columns (Joschko et al. 1993). The

technique of X-ray computed tomography allows one to visualize the open burrow volume present at one time ("snap shot"), whereas nothing can be said about the total soil turnover.

The results of four series of soil column experiments are reported in the following. Two different homogenized soils were used: 1) gleyic phaeozem, FAO (Feuchtschwarzerde, loamy silt, 28% clay); 2) orthic luvisol, FAO (Parabraunerde, loamy silt, 10% clay). The soil was artificially compacted in plastic cylinders (12 cm wide, 30 cm high) to soil bulk densities between 0.93 and 1.54 g/cm^3. They were inoculated with one or two specimen of earthworms (*A. caliginosa, O. cyaneum, L. terrestris*) and kept at 12°C in the dark. Adult individuals were used in all experiments.

After a given time the columns were scanned by means of X-ray computed tomography with a SIEMENS Somatom Plus CT-scanner at the Municipal Hospital, Hildesheim. The columns were subsequently analyzed for their water conducting properties. Since the morphology of burrows determines their function in respect to water movement, special emphasis was laid upon the factors possibly affecting burrow morphology.

B. Computed Tomographic Scanning and Three-Dimensional Reconstruction

In the present study reconstructions of 30 burrow systems are evaluated. Earthworm species and soil bulk density were important factors influencing the results of the three-dimensional reconstructions.

The burrow formation pattern of earthworms is species-dependent (Darwin 1881). Accordingly the three-dimensional reconstructions showed significant differences between earthworm species. The burrows of the anecic species *L. terrestris* were always continuous, with a more or less constant inner diameter. The course of the burrows was not interrupted by soil plugs even at high soil bulk densities (1.5 g/cm^3). This result confirms observations from the field (Graff 1983) and from laboratory experiments (Joschko 1989) according to which *L. terrestris* generally forms permanent continuous burrows.

In contrast to the anecic species *L. terrestris* the burrow patterns of the two endogeic species *O. cyaneum* and *A. caliginosa* were highly dependent on soil bulk density. Burrow pattern, burrow volume as well as the burrow continuity were determined by the initial bulk density of the homogenized soil. Whereas in comparatively loose soil (bulk density 0.9-1.2 g/cm^3) burrows of *O. cyaneum* were highly continuous (Figure 7), the burrow pattern at higher bulk densities (1.4-1,5 g/cm^3) was characterized by a discontinuous arrangement of burrow parts which were often restricted to the upper half of the column (Figure 8).

This pattern was also found in soil columns with *A. caliginosa*. At low soil bulk densities a net of continuous burrows was formed (Figure 9), whereas at high bulk densities the pattern was irregular, discontinuous and sparse (Figure 10). The characteristic burrow pattern found at high bulk density can be

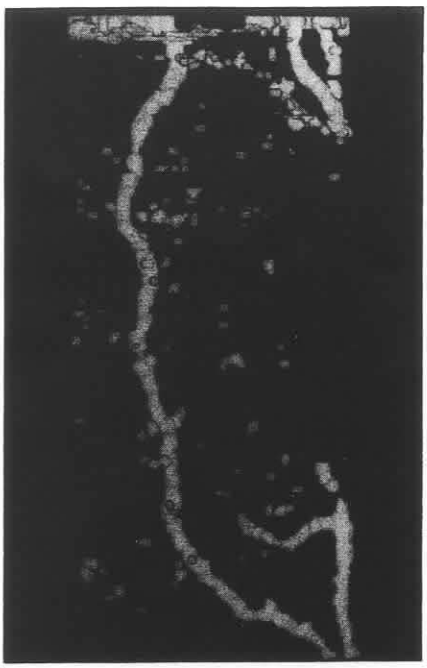

Figure 7. Burrows produced within 50 days by *O. cyaneum* (fresh weight 1.0 g) in gleyic phaeozem artifically compacted to a bulk density of 0.9 g/cm^3.

Figure 8. Burrows produced within 25 days by *O. cyaneum* (fresh weight 3.4 g) in orthic luvisol artifically compacted to a bulk density of 1.5 g/cm^3.

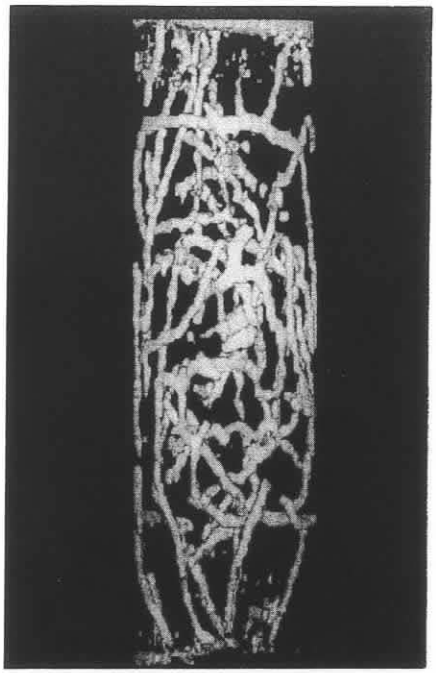

Figure 9. Burrow system produced within 28 days by *A. caliginosa* (fresh weight 1.9 g) in orthic luvisol artificially compacted to a bulk density of 1.2 g/cm^3.

Figure 10. Burrow system produced within 20 days by *A. caliginosa* (fresh weight 2.2 g) in orthic luvisol artifically compacted to a bulk density of 1.5 g/cm^3.

Table 2. Relative burrow volume (expressed in [%] of the total soil volume) / g live weight / day and surface cast production of the endogeic species *A. caliginosa* at the end of the experiment (20-44 days) in relation to soil bulk density.

bulk density [g cm^{-3}]	days	relative burrow volume [%] / g live weight/day	surface casts [g]
1.2	28	0.0459±0.0272	0
1.4	44	0.0056±0.0022	15.6±7.2
1.5	20	0.0062±0.0050	17.4±15.6

explained by the mechanism by which the burrows are formed. At low bulk densities the earthworms can burrow by pushing the soil aside (Graff and Hartge 1974). As a consequence, only few soil plugs (= casts) were found in the course of the burrows in endogeic species or on the soil surface in the surface casting anecic species *L. terrestris*. At high bulk densities in contrast the earthworms "eat" their way through the soil producing casts which are either deposited at the soil surface or inside the soil in pores or elder burrows. Accordingly the amount of casts on the soil surface was significantly higher at high soil bulk densities (Table 2).

The relative effect of the soil bulk density on the burrow pattern was greater than the effect of individual differences between earthworms (Table 2). At low soil bulk density (1.2 g/cm^3) the burrow volume produced per day per g earthworm fresh weight of *A. caliginosa* was significantly higher than the values found at high bulk densities (1.4-1.5 g/cm^3).

C. Measurement of Soil Water Movement

The saturated hydraulic conductivity served as parameter for functionally characterizing the soil fabric under the influence of earthworms. It was measured with a constant head permeameter. The method is described in detail by Joschko et al. (1989).

The comparison between earthworm-worked soil columns and controls without earthworms yielded different results according to the initial bulk density. Whereas in loose soil (0.9-1.2 g/cm^3) the effect of the earthworm burrows on k_f-values was inconsistent in both soils, burrows in more compacted columns (1.3-1.5 g/cm^3) in most cases increased the saturated hydraulic conductivity (Figure 11).

The results of two columns with a species mixture (one specimen of *A. caliginosa*, together with one of *L. terrestris*) indicated that there might be a synergistic effect in increasing saturated hydraulic conductivity. More experiments are needed, however, to substantiate these findings.

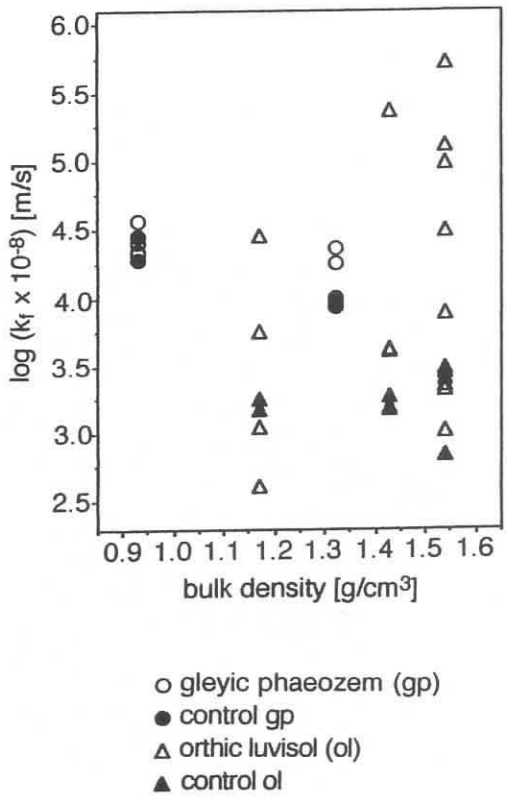

Figure 11. Saturated hydraulic conductivity in columns with earthworm burrows (*L. terrestris*, *O. cyaneum*, *A. caliginosa*) compared to control.

Our results confirm previous experiments with *L. terrestris* and also with *A. caliginosa*, in which an increase of saturated hydraulic conductivity through the action of earthworms was found (Joschko et al. 1989, Joschko et al. 1993). The data indicate that despite the discontinuity of the burrow system at higher soil bulk densities, the burrow function in respect to water movement is considerable.

In order to estimate the contribution of endogeic earthworm channels to water movement in the field a different approach is necessary, however. Ideally burrows in the field are directly studied under natural conditions (Edwards et al. 1989). In addition pot experiments may yield insight into the mechanisms of water movement under unsaturated conditions. In our experiments with planar cuvettes inoculated with earthworms the water movement was directly studied by using fluorescent stains like rhodamine B as tracer (Schrader and Joschko 1991). A downward fingering could be observed along the edges of continuous earthworm burrows. The results thus indicated that under unsaturated conditions

the drilosphere (sensu Bouché 1975), i.e. the adjacent soil of the burrows, consisting of smeared casts and compacted soils, provides preferential pathways for the percolating water.

D. Correlation Between Burrow Morphology and k_f

In the soil columns two different hydraulic situations are to be distinguished. When the burrows are continuous macropores leading through the whole column they comprise preferential flow paths. In this case burrow length proved to be a functionally important parameter. This was estimated by measuring gypsum filled burrows which were preferentially produced along the walls of the plastic cylinder (Joschko et al. 1993). Such continuous burrows are characteristic for endogeic earthworm species at low soil bulk densities (see above).

In case of discontinuous burrows which are produced at higher soil bulk densities additional parameters have to be searched for. Potential functionally important parameters were derived from the burrow pixel distributions of single columns where the numbers of burrow pixels in each horizontal slice were summed up (Joschko et al. 1993). The following calculations refer to a series of 10 columns with orthic luvisol of a bulk density of 1.5 g/cm^3. In order to gain insight into the function of the burrow system several parameters were tested for their correlation with the k_f-values derived from the respective columns.

The definition of possible parameters was stimulated by the work of Kretzschmar who recommended to quantify the "matrix distance" for functional analyses of the earthworm burrow systems (e.g. Monestiez and Kretzschmar 1989). In our data no correlation was found between the total number of burrow pixels versus k_f-values. However, a negative exponential relationship ($r=-0.597$) between the distance below the deepest burrow in a column and the respective k_f-value could be established. A similar result has been described earlier with respect to burrows of *L. terrestris* (Joschko et al. 1989).

Due to the discontinuity of the burrows the matrix between burrow parts has to be considered as well. Therefore the ratio of the number of rows with burrow pixels to the total number of vertical pixel rows per column was defined as a measure for the vertical continuity of the burrows. In case of a continuous macropore the ratio is 1. The correlation coefficient was $r=0.76$ when rows with pixel numbers of 10 and lower were excluded (Figure 12). The results suggest that the vertical continuity of the burrows after exclusion of low pixel numbers (="noise") is a functional important parameter for the water movement under saturated conditions.

This approach however only considers a one-dimensional flow by summing up all values in a horizontal plane. In future a more realistic analysis of the functioning of the burrow systems would have to apply a three-dimensional flow model. A promising approach was described by Thoma and Priesack (1991), where a three-dimensional simulation model was used. The technique of X-ray

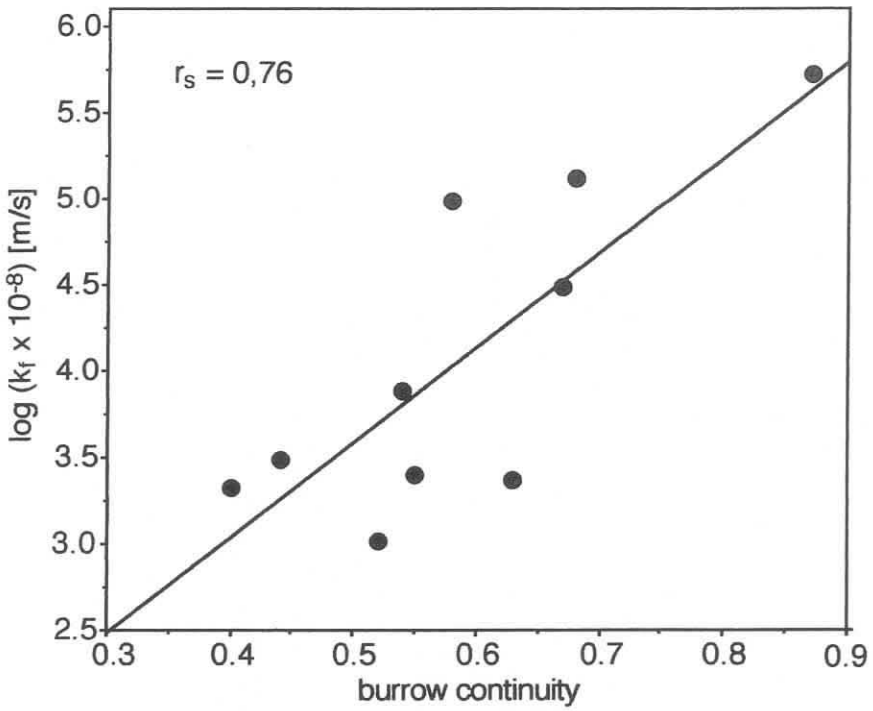

Figure 12. Correlation (Spearman's rank correlation) between vertical burrow continuity and saturated hydraulic conductivity in orthic luvisol, artifically compacted to a bulk density of 1.5 g/cm^3.

computed tomography yields a three-dimensional data matrix of macropore distribution which can be incorporated into simulation models. In order to approach realistic field conditions the simulation of water movement under unsaturated conditions should also be investigated.

E. Assessment of Burrow Stability

The stability of burrows has several aspects. The rearrangement of the burrows with time initiated by the earthworm itself is one measure of stability.

When earthworms are inoculated into soil they start burrowing. In planar glass cuvettes a steady increase of burrow length with time was found (Figure 13). The rate of burrow length increase was dependent on earthworm species. 47% of the total burrow length were plugged with casts in both species at a soil bulk density of 1.3 g/cm^3.

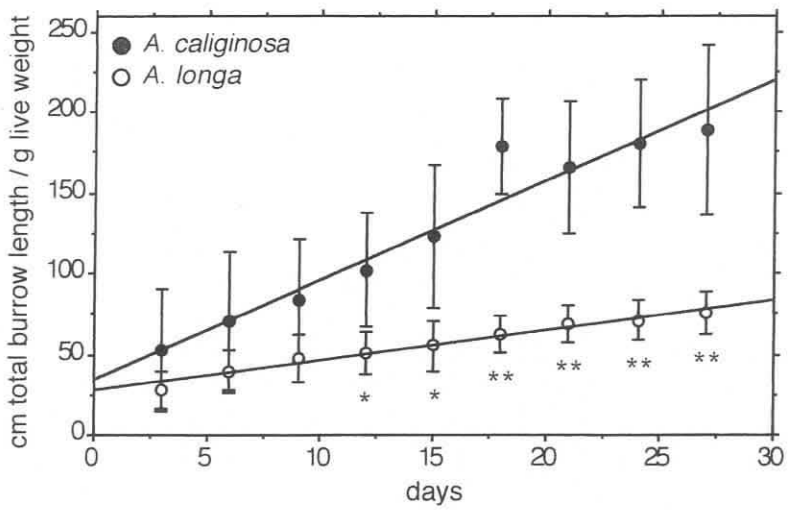

Figure 13. Temporal development of the increasing total burrow length. Arithmetic means (±SD) for n=4 vessels with 2 individuals each. *P≤0.05 and **P≤0.01. *A. caliginosa*: y = 6.23x + 34.25; r^2=0.938; *A. longa*: y = 1.90x + 27.25; r^2=0.978.

With X-ray computed tomography the changes of the three-dimensional burrow pattern with time may be studied as well. The burrows of the endogeic species *O. cyaneum* turned out to be rather unstable when soil columns with burrows were scanned after 92 days compared to the burrow pattern at days 14 and 21. The results suggest that the vertical burrow pattern may change drastically within weeks; in one case the burrows shifted almost completely from the upper to the lower half of the column. In respect to the horizontal pattern the burrows were at first restricted to the outer walls of the cylinders. From there the burrows penetrated into the column core (Joschko et al. 1993).

The stability toward water is reflected in the course of the k_f-values with time during the measurements. In nearly all soil columns the k_f-values decreased with time, indicating a relatively low stability of the soil. But the decrease of the values was only slight. This result indicates a relatively high stability of the burrows compared to artificial macropores which generally are quickly destroyed due to slaking. Earthworm burrows in contrast more or less preserved their shape during the saturation and measurement procedure.

IV. General Discussion

The study of the thin sections revealed separation and compaction processes during the gut passage of soil in *A. caliginosa*. The separation resulted in fragmental microaggregates and clay coated silt grains which showed an open arrangement. Marinissen and Dexter (1990) pointed out that besides organic matter, microbial activity and drying-rewetting cycles, the amount of microaggregates is important for the stability of aggregates. But these above mentioned processes do not happen to the whole ingested soil as undestroyed aggregates showed. Altemüller and Joschko (1992) found parts in the casts of *L. terrestris* which were also not rearranged through the intestinal passage. These observations are in contrast to Barois et al. (1993). They found a complete destruction of the soil structure with dispersion of all particles in the foregut of *Pontoscolex corethrurus*.

The reduction of microorganisms in the gut may be interpreted as partial digestion of microflora. This observation is in agreement with former microbiological studies on bacteria (Dawson 1947), fungi (Domsch and Banse 1972) and algae (Atlavinyte and Pociene 1973). Some species were also reduced or no longer detectable in fresh casts. More recently, Kristufek et al. (1992) reported a significant decrease of microorganisms from foregut to hindgut in *A. caliginosa*. Altemüller and Joschko (1992) detected a higher number of bacteria in casts of *L. terrestris* compared to the surrounding soil by fluorescence microscopy. Such differences may be due to the different food selection of geophagous (*A. caliginosa*) and detritivorous (*L. terrestris*) species which has also consequences for the stability of casts.

The stability of aggregates depends primarily on the amount and disposition of organic matter (Tisdall and Oades 1982). Furthermore, soil aggregates become stable by age-hardening due to numerous drying-rewetting cycles (Dexter et al. 1988). With exception of the natural aggregates all the materials of the present study as there are casts, burrow-wall material and remolded aggregates can be seen as newly formed material. The molding procedure, artificially or worm caused, destroyed the linkages between soil particles to a great extent. But only in the worm-worked soil could the linkages be rebuilt mostly because of the enrichment with organic matter and drying before the measurements. From this point of view the binding forces of clay-polyvalent cation-organic matter linkages as defined by Shipitalo and Protz (1989) are presumed to operate. Shipitalo and Protz (1988) were the first to point out the reduced water stability of earthworm casts kept moist during the first weeks after egestion. The results presented here show that even drying of 4 weeks old casts did not enhance the stability compared to the surrounding soil. This may also be connected with the soil texture. The soil used here contained only 13% clay.

Polysaccharides may be linked to clay minerals forming complexes of high tensile strength (Chenu and Guérif 1991). But this is not valid in general. The best conditions for a stabilizing effect are high molecular weight and a linear or

helical solution conformation (Hayes 1983). Martens and Frankenberger (1992) could not correlate the polysaccharide concentrations of soil samples with water-stable aggregation or bulk density. They pointed out the heterogeneity of polysaccharide properties produced by different strains of bacteria. The results of the present paper indicate a similar case since the polysaccharides as well as their monomeres were not correlated with water stability or tensile strength. Conformation and arrangement of the monomeres seemed to be more important than their concentrations. According to a review by Anderson (1991) hydrophobic substances like lipids and adhesion mechanisms of microorganisms are also significant for the stability of soil aggregates.

The influence of endogeic earthworms on the soil fabric depends on the original status of the soil fabric itself. The relative amount of soil which is processed by the earthworm during its digestion and subjected to separation and compaction processes as described above is higher at high bulk densities of the soil. Analogous results have been described for the anecic species *L. terrestris* (Joschko 1989). At low soil bulk densities separation and compaction processes are also found but they refer to another dimension of soil. In these cases the soil around the earthworm is compressed when it moves through the substrate by pushing it aside.

The physical conditions as a major factor in determining the burrowing behavior of endogeic earthworms should be studied more closely. With X-ray computed tomography a tool is now available which allows to assess discontinuous burrow systems in three dimensions as well as their alteration within time. The technique may also be applied to soil monoliths taken from the field. A lack of information still exists in respect to the quantification of the three-dimensional burrow pattern.

The vertical continuity of the burrow system which is affected by the soil bulk density and dependent on earthworm species determines the function of the burrows in respect to water movement under saturated conditions. Fingering along burrow walls when water is moving under unsaturated conditions points to another case (Schrader and Joschko 1991). Finally for the assessment of the influence of earthworms on water movement their potential to increase infiltration by penetrating crusted soil surfaces has to be considered as well (Roth and Joschko 1991).

Earthworms may influence transport processes through the construction of burrows of various shape, through perforations of crusted soil surfaces, through the production of water flow impeding casts and through compaction of the soil when moving through the substrate. Whether and to what extent these mechanisms are manifested depends on the earthworm species and on physical soil conditions like soil bulk density. The relative importance of these mechanisms for the soil water movement is a function of the soil water content and the amount of water applied on the soil surface. X-ray computed tomography may yield the morphological data necessary to quantify, model and understand these different modes of water transport through soil which is under the influence of earthworm activity.

V. Conclusions and Recommendations

(1) During intestinal passage in *A. caliginosa* clay material is separated in fragmental microaggregates. Microorganisms are effectively digested during this passage. The fabric of gut contents should be studied more closely with respect to other soil material and earthworm species in order to gain information on cast formation.
(2) The differences in food selection between *L. terrestris* and *Aporrectodea spp.* resulted in greater stability of the casts of *L. terrestris* due to a higher organic carbon content. Tensile strength and organic carbon were positively correlated whereas tensile strength and calcium carbonate were negatively correlated. It is supposed that calcium carbonate is involved in the formation of organic bonding agents such as Ca-humates. The stability of dried 4-week old casts was slightly less than the bulk soil but enhanced compared to remolded aggregates. However, the stability is not correlated with the content of polysaccharides and their monomeres. Their composition and conformation may be more important. Further study should be focused on this aspect.
(3) The three-dimensional burrow pattern of earthworms (*L. terrestris*, *A. caliginosa, O. cyaneum*) is highly variable, depending on earthworm species, soil bulk density and time. In functional studies this variability in time and space has to be taken into consideration. In soil column experiments the X-ray computed tomography in combination with techniques for three-dimensional visualization allows the non-destructive assessment of burrow systems and their variation in time.
(4) The functional relationship between earthworm burrow systems and water movement comprises several aspects. Important parameters are the vertical burrow continuity, the shape of the soil surface and the physical characteristics of the soil around the burrows, i.e., the "drilosphere". A comprehensive conceptual and functional model of the consequences of earthworm activity for soil water transport processes is still required.

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